Drs. Bing
Gardner
Sommers

CARDIOVASCULAR #889R1

THE COUNCIL FOR TOBACCO RESEARCH – U.S.A., INC.

110 EAST 59TH STREET
NEW YORK, N. Y. 10022
(212) 421-8885

Application For Renewal of Research Grant

(Use extra pages as needed)

First Renewal [X] Second Renewal [ ] Date: 07/10/73

1. Principal Investigator (give title and degrees): Timothy J. Regan, M.D.
Professor of Medicine
Director, Division of Cardiovascular Disease

2. Institution & address: College of Medicine & Dentistry of New Jersey-New Jersey Medical School
100 Bergen Street
Newark, New Jersey 07103

3. Department(s) where research will be done or collaboration provided: Division of Cardiovascular Disease
Department of Medicine
New Jersey Medical School

4. Short title of study: Variables affecting the cardiovascular responses to chronic smoking.

5. Proposed renewal date: January 1, 1974 to December 31, 1974

6. How results to date have changed earlier specific research aims:

In these chronic studies the animals have not been in the program sufficiently long to warrant a change in our specific research aims.

7. How results to date have changed earlier working hypothesis:

Refer to item # 6.

8. Any additional facilities now required? Describe briefly: NO.

9. Any changes in personnel? Append biographical sketches of new key professional personnel:

NO.

10. Append outline of experimental protocol for ensuing year.

11. List publications or papers in press resulting from this or closely related work. (append reprints or manuscripts not previously sent).

a. Regan, T.J., and Moschos, C.B.: Effects of a chronic smoking program upon clotting and fibrinolysis in dogs. Presented at the Third Workshop Conference on Tobacco and Health, American Medical Association Education and Research Foundation, Newport Beach, Calif., 1972. (Abstract.)

b. Ahmed, S., Levinson, G.E., Moschos, C.B., Oldewurtel, H., and Regan, T.J.: Effect of smoking nicotinized and nonnicotinized cigarettes on systolic time intervals. Clin. Res. 20: 359, 1972. (Abstract.)

c. Ahmed, S.S., Moschos, C.B., and Regan, T.J.: Cardiovascular effects of chronic smoking in beagles. Circulation 45, 46: II-103, 1972. (Abstract.) Presented at the 45th Scientific Sessions, American Heart Association, Dallas, Texas, 1972.

d. Ahmed, S.S., Moschos, C.B., Sethi, V., Ettinger, P.O., and Regan, T.J.: Comparative cardiovascular physiology of beagle and mongrel dogs. Physiologist 15: 69, 1972. (Abstract.) Presented at the 23rd Annual Fall Meeting, American Physiological Society, University Park, Pa., 1972.

12. Summary progress report (append in standard form as separate document, unless recently submitted)

10. Experimental protocol for ensuing year.

The chronic smoking program will be carried out in litter mate beagles. After more than two years experience we have found this species quite tolerant of the chronic smoking program. The technique of chronic tracheostomy under anesthesia as described by Cahan and Kirman (3) is employed. After ten days of healing, the animals begin cigarette smoking by replacing the regular tracheostomy tube with a teflon tube coupled with latex tubing to the smoking machine which regulates the duration and volume of inhaled smoke. Two weeks are usually needed for the animal to adjust to the initial reaction and to smoking voluntarily. Both control and experimental animals will have tracheostomy and will be maintained in the same environmental conditions. Routine monitoring of weight, hematocrit and serum proteins is performed throughout the study. In addition, at intervals of three months, blood will be taken to assess clotting proteins, platelets and plasma lipids. Arterial blood pressure will be monitored in the awake but relaxed animal, to evaluate our previous observations of hypertension in the anesthetized beagle in a chronic smoking program. The animals will also have studies of ventricular conduction by high frequency precordial EKG (4) and ventricular function from the ejection times (5). These experiments will consider the following variables: the duration of smoking, the influence of high lipid diet and the effects of combined ethanol use.

Litter mate beagle dogs, one to two years of age, will be used. Each group will be divided into a smoking and nonsmoking group. It is intended to observe them at least two to three years. Group I: eight animals will be maintained on a standard canine diet without smoking and eight will smoke seven cigarettes per day with the same diet. Group II will consist of eight dogs placed on the standard diet plus 36% of calories as ethanol; the other half of the group will receive the same regimen combined with smoking.

a. Clotting and fibrinolytic studies: The following is a summary of the studies on coagulation and fibrinolytic activity carried out in our currently ongoing chronic smoking program.

1) Whole blood clotting time (Lee and White) in glass and plastic tubes in duplicate.

2) Partial thromboplastic time (Hicks and Pitman, Brit. J. Haematol. 3: 227, 1957.

3) Platelet counts--direct (Brecker, C., and Cronkite, E.J., J. Appl. Physiol. 3: 365, 1950).

4) Platelet adhesiveness (Saltzman, E.J., J. Clin. Med. 62: 74, 1963).

5) Plasma fibrinogen levels (Quick, A.J., Hemorrhagic Disease. Phila., Lee and Faberger, 1957, pp. 426-439).

6) Fibrinolytic activity by Euglobulin lysis time (Van Kaulla, K., and Schultz, R., Amer. J. Clin. Pathol. 29: 104, 1958) and on unheated bovine fibrin plates (method of Astrup and Muller, as modified by Holemans and Robers, J. Lab. Clin. Med. 64: 778, 1964).

In addition to these parameters, hypercoagulable state or thrombogenicity will be evaluated by kinetic studies of platelet and fibrinogen. Thus, the survival rates of ^{51}Cr tagged platelets (6) and ^{125}I tagged fibrinogen (7) will be determined from their disappearance rates in sequential samples taken after administration of the tracers (7). In addition, platelet function will be evaluated by estimating platelet aggregation. Platelet rich plasma aliquots will be exposed to standard doses of ADP, collagen or epinephrine, recording aggregation patterns by means of an aggregometer (8, 9). At the conclusion of the study, samples will be taken from the artery and coronary sinus for evaluating of clotting and fibrinolytic activity as well as evaluation of thrombogenicity by means of the Wessler technique. The myocardial and renal uptake of fibrinogen will be assessed. If indicated for localization, autoradiography will be performed.

b. Morphology: Since we have observed apparent accumulation of triglyceride in myocardial cells in chronic smoking beagles, myocardial speciments will be obtained by a catheter biopsy technique for light and electron microscopic examination at six months intervals. Following sacrifice of the dog or in case of sudden death, tissue specimens from heart muscle, coronary arteries, conduction system as well as aorta, will be subjected to extensive morphologic examination and the results will be correlated with those obtained from the hemodynamic and metabolic studies. Specimens of coronary artery, conduction bundle and myocardium are to be fixed in 10% neutral buffered formalin for routine microscopy. Sudan IV preparations are prepared on formalin fixed cryostat-sectioned material. Remaining tissues are processed and stained with hematoxylin eosin, Alcian Blue, PAS, Gormori's aldehyde-fuchsin, Van Gieson elastica and trichrome, Specimens for electron microscopy are cut into small sections fixed in cold-buffered glutaraldehyde, post-fixed in osmium, exposed to lead and uranyl acetate and then imbedded in epon. Sectioning is done on a Porter-Blum ultramicrotome and electron microscopy performed on a Siemens Elmskop I.

c. Ventricular function: The smoking periods will be concluded after 12, 24 or 36 months. Each dog will be anesthetized for hemodynamic studies with chest intact. The parameters include left ventricular systolic pressure and its first derivative, end-diastolic pressure and volume, cardiac output by the indicator dye dilution technique at rest and during afterload or preload increments, using angiotensin or rapid intraventricular infusion of normal saline; calculation of stroke volumes and stroke work as well as determination of ventricular contractility by the Frank-Levinson index (10). In addition, conduction in the His-Purkinje system and ventricular wall will be measured (11).

d. Myocardial metabolism: Coronary blood flow will be assessed by the ^{85}Kr clearance technique (12) before and during tachycardia induced with atrial pacing. Myocardial production of lactate (13) or leakage of potassium into the coronary sinus will be evaluated as an index of myocardial ischemia. Electronmicrographs will supplement the evaluation of ischemia. In addition, after a suitable recovery period, the heart will be cold-arrested and the transmural distribution of potassium, sodium, triglyceride and free fatty acid, since altered myocardial content has been observed during ischemia or catecholamine infusion (14).

REFERENCES

1) Regan, T.J.: Ethyl alcohol and the heart. Circulation 44: 957, 1971.

2) Regan, T.J., Khan, M.I., Ettinger, P.O., Jesrani, M.U., Lyons, M., and Oldewurtel, H.A.: Myocardial function and metabolism in the well nourished chronic alcoholic animal. Circulation 44: II-49, 1971. (Presented at the 44th Scientific Session, American Heart Assn., Anaheim, Calif., 1971).

3) Cahan, W., and Kirman, D.: An effective system and procedure for cigarette smoking by dogs. J. Surg. Res. 8: 567, 1968.

4) Flowers, N.C., Horan, L.G., Tolleson, W.J., and Thomas, J.R.: Localization of the site of myocardial scarring in man by high-frequency components. Circulation 40: 927, 1969.

5) Weissler, A.M., Peeler, R.G., and Roehill, W.H., Jr.: Relationships between left ventricular ejection time, stroke volume, and heart rate in normal individuals and patients with cardiovascular disease. Am. Heart J. 62: 367, 1961.

6) Aster, R.H.: Platelet sequestration in man. J. Clin. Invest. 43: 843, 1964.

7) Takeda, Y.: Studies of the metabolism and distribution of fibrinogen in healthy man with autologus ^{125}I-labelled fibrinogen.

8) Borne, G.: The aggregation of blood platelets. J. Physiol. 168: 178,1963.

9) Mustard, J.F., Hegardt, B., Rowsell, C., and McMillan, R.L.: The effect of adenosine nucleotides on platelet aggregation and clotting time. J. Lab. Clin. Med. 64: 548, 1964.

10) Frank, M.J., and Levinson, G.E.: An index of the contractile state of the myocardium in man. J. Clin. Invest. 47: 1615, 1968.

11) Ettinger, P.O., Khan, M.I., and Regan, T.J.: A catheter electrode technique for study of left ventricular conduction. J. Appl. Physiol. 28: 519, 1970.

12) Harman, M.A., Markov, A., Lehan, P.H., Oldewurtel, H.A., and Regan, T.J.: Coronary blood flow measurements in the presence of arterial obstruction. Circ. Res. 19: 632, 1966.

13) Regan, T.J., Markov, A., Oldewurtel, H.A., and Burke, W.M.: Myocardial metabolism and function during ischaemia: Response to l-noradrenaline. Cardiovasc. Res. 4: 334, 1970.

14) Regan, T.J., Markov, A., Khan, M.I., Jesrani, M.U., Oldewurtel, H.A., and Ettinger, P.O.: Myocardial ion and lipid changes during ischemia and catecholamine induced necrosis: Relation to regional blood flow. In: Myocardiology: Recent Advances in Studies on Cardiac Structure and Metabolism. E. Bajusz, and G. Rona, editors, University Park Press, Baltimore, London, Tokyo, vol. 1, 1972, pp. 656-664.

13. Budget for the coming year:

A. Salaries (give names or state "to be recruited")	% time	Amount
Professional (give % time of investigator(s) even if no salary requested)		
T.J. Regan, M.D.	15%	xxxxxx
C.B. Moschos, M.D.	20%	xxxxxx
M.M. Lyons, M.D.	15%	xxxxxx
S.S. Ahmed, M.D.	30%	xxxxxx
G. Manskopf, M.D.	20%	xxxxxx
H. Oldewurtel	10%	xxxxxx
Technical		
F. Herdman	100%	6,736
R. DeSantis (diener)	75%	7,200
Pathology technician	45%	5,013
		18,949
Fringe benefits (17%)		3,221
	Sub-Total for A	22,170

B. Consumable supplies (by major categories)

Animals: 30 litter mate beagles @ $108 ea.	3,240
Animal maintenance, $1/dog/day	10,950
Smoking apparatus, tracheostomies, cigarettes	275
Glassware, syringes	350
Reagents for coagulation & biochemical analyses	610
Special diet (high lipid)	780
Isotopes 125-I, 51-Cr, 85-Kr)	890
Sub-Total for B	17,095

C. Other expenses (itemize)

Travel: domestic, attend scientific meetings	300
Sub-Totall for C	300
Running Total of A + B + C	39,565

D. Permanent equipment (itemize)

Sub-Total for D	
E	5,935
Total request	45,500

E. Indirect costs (15% of A+B+C)

14. Other sources of financial support:

List financial support from all sources, including own institution, for this and related research projects.

CURRENTLY ACTIVE

Title of Project	Source (give grant numbers)	Amount	Inclusive Dates
Effects of nicotine on blood coagulation, myocardial blood flow and function.	AMA Education & Research Foundation	50,000	05/72-04/74
Alcoholism and diseases of the heart.	NIMH-USPHS- MH17007-4	132,445	05/72-04/75
Myocardial function and metabolism in chronic diseases.	RFP NHLI-72-2970 - USPHS	658,065	01/73-12/77

PENDING OR PLANNED

Title of Project	Source (give grant numbers)	Amount	Inclusive Dates

It is understood that the investigator and institutional officers in applying for a grant have read and accept the Council's "Statement of Policy Containing Conditions and Terms Under Which Project Grants Are Made."

Principal investigator

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Signature Timothy J. Regan Date 07/10/73

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Responsible officer of institution

Typed Name Harold A. Kaminetzky, M.D.

Title Dean of Medicine

Signature Harold A. Kaminetzky Date 7/16/73

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